

LPS and GM-CSF cause Internalization of NADPH oxidase to an Agonist-Responsive, Exocytic Storage Compartment in Macrophages

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Mature, resting macrophages express the superoxide-producing NADPH oxidase on the cell surface. Here we report that, unexpectedly, stimulation of macrophages with lipopolysaccharide (LPS) or GM-CSF quantitatively redistributes cyt b₅₅₈ (the membrane-integral, catalytic core of NADPH oxidase) from the plasma membrane to an intracellular storage compartment composed of a numerous, small (≤ 100 nm) vesicles. Redistribution was clathrin-dependent as cyt b₅₅₈ localized to clathrin-coated pits, and internalization was inhibited by a dominant negative mutant of the clathrin-coated pit-associated protein Eps15. In response to phagocyte activation, the cyt b₅₅₈-containing storage vesicles were recruited efficiently to phagosomes, and further, the known secretagogue ATP and the proinflammatory agents TNF- α and CD40L induced exocytosis of cyt b₅₅₈-containing storage vesicles to substantially increase the cell surface expression levels of cyt b₅₅₈. Exocytosis was followed by re-internalization of cyt b₅₅₈. In conclusion, NADPH oxidase in GM-CSF or LPS-stimulated macrophages occupies an intracellular, secretory storage compartment, which allows rapid agonist-induced redistribution of superoxide production in the cell. Therefore in theory at least, extrinsic signals in a local, inflammatory environment dictate the subcellular distribution of NADPH oxidase activity in macrophages, and this in turn is expected to determine the species and magnitude of reactive oxygen species released to surrounding tissues.